Complex Measurements of Fluorescence and Speckle Contrast in Laboratory Mice during Pancreas Ischemia Modeling

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Abstract

The paper describes the experiment at pancreas ischemia model carried out by combining the methods of fluorescence spectroscopy and laser speckle contrast imaging in laboratory mice. The measurement was performed before and after ischemia to evaluate the relationship between the changes of blood flow and metabolic activity. The results showed high positive correlation between fluorescence intensity at excitation wavelength of 365 nm and speckle contrast. At the same time, 450 nm fluorescence intensity and speckle contrast showed high negative correlation as well as two kinds of fluorescence maxima. The proposed approach seems promising for further studies and future clinical testing.

Keywords: fluorescence spectroscopy, speckle contrast, mice, pancreas, ischemia

I Introduction

Currently, acute destructive pancreatitis is one of the urgent problems in abdominal surgery due to its high complications probability and mortality. Average mortality rate of pancreatitis is 20-45%; however, it can reach up to 85% in infected pancreatic necrosis and 100% in fulminant form[1].

The main factors of acute pancreatitis progression include microcirculation disorders, ischemic reperfusion injury and the transition from apical apoptosis to necrosis. One of the most important factors among them is ischemic-reperfusion injury of the pancreas. Violations of microhemodynamics is leading mechanism, which triggers progression of pathological processes in pancreas tissue[2,3]. This causes tissue anoxia, which leads to cell metabolism disorders.

One of the main problems surgeons face in this pathology is lack and low quality of diagnostic information in obtained clinical condition during the surgical operations. This problem is even more relevant to minimally invasive surgical operations. In last decade, this approach became more widespread for diagnosing and surgical treatment of the patients with the pathologies of abdominal organs. Compared with open surgery, minimally invasive surgery allows one to decrease the operative morbidity and mortality, as well as to lower the recovery time of the patient and rehabilitation cost[4], because the challenges of the intraoperative collection and processing of the diagnostic information in real time are not solved well yet. Informative rapid diagnostic methods will allow the surgeon to timely assess the patient's condition during surgical operations and choose the right tactics. Therefore, development and verification of novel diagnostic techniques and criteria for visualization and analysis of inflamed and necrotic tissues in minimally invasive surgery is rapidly growing field of research.

At present, a gold standard for tissue recognition is a histological examination of samples obtained by biopsy. This method is used as a reference for other techniques and for accurate diagnosing. However, obtaining results takes much time and the method itself may cause several types of complications. Existing widespread diagnostic methods allows either obtain the information about the

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pathological state of tissue later or obtain intraoperative information about anatomical and morphological features rather than morphological changes in tissue. The development and implementation of the method combining the advantages of both approaches is necessary for improving the quality of diagnostics[5].

Possible solution is optical non-invasive methods, which are rapidly applied in more and more areas, including practical medicine[6]. This approach includes various spectroscopic and imaging techniques for studying biological tissues *in vivo* in real time without invasive acquiring the sample of this tissue. These methods allow to evaluate metabolic processes, chromophores content, blood perfusion and oxygenation[7]. Optical methods can increase the prevalence and effectiveness of minimally invasive abdominal interventions in clinical practice by providing additional diagnostic information for surgeon in real time.

One of the most widespread optical spectroscopic methods for *in vivo* diagnostics is fluorescence spectroscopy (FS). The method is based on the excitation of fluorescence of endogenous and exogenous tissue fluorophores with UV or visible optical radiation to record the fluorescence emission spectra. The rate of metabolic processes in biotissue manifests in changes of concentration of certain endogenous fluorophores involved in tricarbonic acid cycle. In particular, the reduced form of NADH coenzyme has a fluorescence intensity maximum at a wavelength of 490 nm when excited with 365 nm radiation. Oxidized FAD has a maximum of fluorescence intensity in the range of 520-540 nm when excited with 450 nm radiation[8].

The metabolic processes depend on proper tissue oxygen supply delivered by blood. The information about blood microcirculation in organs tissue is important to determine normal tissue and inflammatory or necrosis processes during the surgery. Therefore, many optical techniques are developed for this purpose as well as for studying of biochemical reactions closely related to blood supply disorders, at the moment, the most effective methods are diffuse reflectance spectroscopy[9] and imaging[10], laser Doppler imaging[11] and laser speckle-contrast imaging (LSCI)[12].

LSCI method is based on the properties of laser radiation. When laser radiation falls on the inhomogeneous surface random interference of coherent light occurs, which creates a randomly changing picture of the intensity, known as speckle. Temporal and spatial statistics of speckle patterns provide information on the motion of scattering particles.

As the metabolic activity and blood microcirculation are closely interrelated, it makes sense to study both of these parameters together. Multimodal approach seems more promising for complex diagnostics of different aspects of pathological processes in tissues[13,14].

The aim of the work is to study the possibility of combined application of optical spectroscopic and imaging techniques for multimodal diagnosing of the pancreas tissue state.

II Materials and Methods

The experiment was performed on model mice. The experiment was approved by Ethics committee of Orel State University (record of the meeting №10 of 16.10.2017).

The equipment included two setups implementing FS and LSCI methods each (Fig. 1, 2).

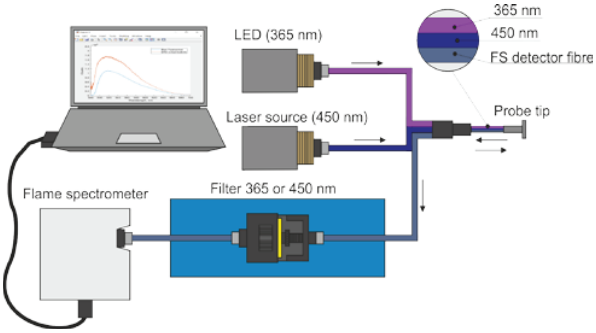


Figure 1. The schematic illustration of FS setup

The first setup implements FS method. A 365 nm UV light source (LED) and 450 nm blue light source (laser diode) were used to excite the emission of NADH and FAD coenzymes showing the metabolic activity. FS channel has been composed of two emitting and one collecting fibers. The fibers were combined in a single fiber optical probe. The optical filters were used to exclude backscattered source radiation and to obtain only fluorescence spectra.

Collected light was recorded by a CCD spectrometer in the range of 350-820 nm (FLAME, Ocean Optics, USA). A personal computer was used to control the system and

automate the data acquisition process. The data processing has been performed using custom-developed software in MATLAB software.

Figure 2 shows the scheme of LSCI setup.

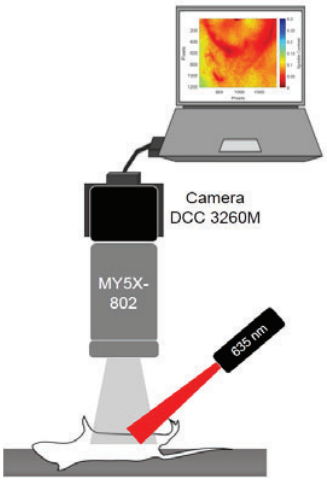


Figure 2. The schematic illustration of LSCI setup

10 mW laser source working at 635 nm wavelength (Edmund Optics Inc., USA) was used to illuminate the object. CMOS-camera DCC 3260M (Thorlabs, Inc., USA) with 1936×1216 pixels and 5.86 μm pixel size combined with 34 mm Mitutoyo Plan Apochromat Objective MY5X-802 (Thorlabs, Inc., USA) recorded raw speckle images. The obtained images were processed with a custom-developed algorithm in the offline mode. The standard spatio-temporal algorithm has been used for speckle contrast images obtaining. The calculation of the average speckle contrast of the image was performed using the Eq. 1:

$$K = \left\langle \frac{\sigma_N}{\langle I \rangle_N} \right\rangle_k , \qquad (1)$$

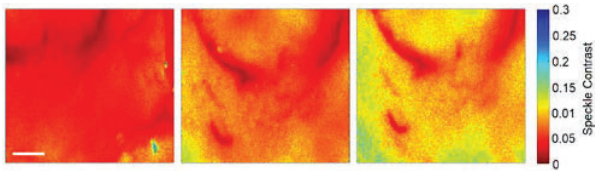
where $\langle\ \rangle$ – the symbol of averaging; N – the window of averaging NxN (N=7); k – the number of consecutive frames (k=20); $\langle I \rangle_N$ – average intensity in the window NxN; σ_N – standard deviation in the window NxN.

Experimental studies were performed on clinically healthy male Balb/c mice. Mice were obtained from the "Andreevka" vivarium FSBI SCBT FMBA of Russian Federation. Before the transfer to the clear zone of the laboratory and the experiment, the animals were kept in quarantine for 14 days. When placed in quarantine, the veterinarian conducted a primary assessment of the animals condition with the introduction of the examination results in the relevant statement and then examined animals daily. The basic rules of maintenance and care corresponded to the standards of the sanitary rules for the arrangement, equipment and maintenance of experimental biological clinics and in the guide "Laboratory animals"[15] and GLP principles.

During the study, mice were anesthetized with Zoletil 100 (Vibrac, France) at standard dose. Each animal was fixed on a special platform in the position on the back. A transverse laparotomy procedure was performed; the operative access was made to the upper part on the back wall of the abdominal cavity in the retroperitoneal space. After that, the complex of organs containing the pancreas was carried out. The body of the pancreas was isolated and ligature from polyester thread was placed. In addition, a cotton swab soaked in a 0.9% solution of sodium chloride was placed in the operating field. Then, the animal was placed under the optical system to visualize the area and record the frame sequences. After that, the fiber probe was placed in the middle of visualized area and three pairs of fluorescence spectra were registered. The registration of images and spectra was made before ligating and after 1, 6, 11 .. 66 min after ligating of pancreas vessels.

III Results and Discussion

The setup showed in Figure 2 was used to record the sequence of frames in mice. For each stage, a sequence of 20 frames was recorded. After image processing with the space-time algorithm spectral contrast images were obtained (Fig. 3).



a) b) c)

Figure 3. Speckle images from mice before (a), 26 min (b) and 56 min (c) after ligating pancreas. Scale bar equals 2 mm.

The average contrast ratio of the speckle image in the area of 100x100 pixels was calculated using the Eq. 1 to acquire average speckle contrast values (Fig. 4).

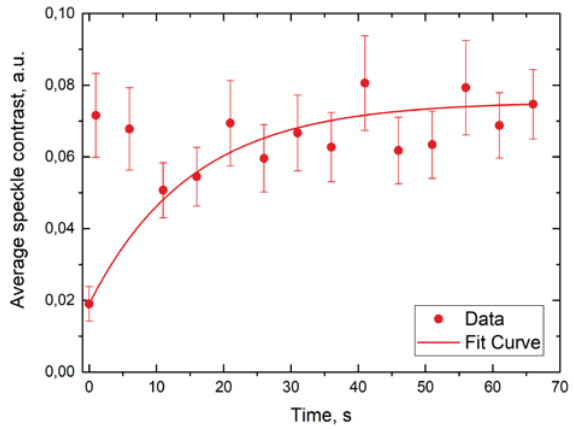


Figure 4. Average speckle contrast value of 100x100 pixels area during ligation process.

Figure 4 shows gradual increasing of speckle contrast value after ligating the pancreas, which indicates the slowing of blood flow and development of tissue ischemia. Slow change of speckle contrast and its oscillations indicate that the venous blood flow still occurred as the blood left the vessels remained without arterial supply.

The results of measurements by FS method are shown in Figure 5.

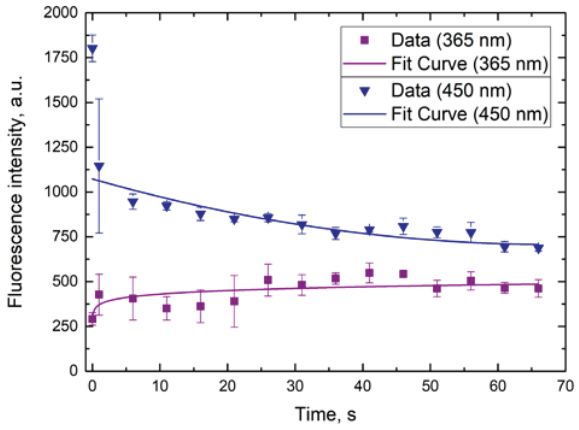


Figure 5. Average fluorescence intensity maxima measured during the experiment.

Figure 5 shows that intensity of fluorescence excited by 365 nm radiation increases as well as average speckle contrast. At the same time, the fluorescence excited by 450 nm radiation greatly decreased after ligation and continued to drop slowly. Such pattern indicate that progressing tissue ischemia has led to accumulation of NADH, which is associated with a lack of oxygen. Meanwhile, the consumption of FAD increases which manifests in its fluorescence decrease. These effects are a sign of hypoxia and poor blood supply of pancreas tissue.

The Pearson correlation coefficient was calculated to prove the interconnection of data obtained. The results of analysis are presented in Table 1.

Table 1. Pearson correlation between fluorescence intensity maxima and average speckle contrast.

Data Sample 1	Data Sample 2	Pearson Correlation Coefficient for p≤0,01
Average speckle contrast	365 nm fluorescence	0.6908
365 nm fluorescence	450 nm fluorescence	-0.67575
450 nm fluorescence	Average speckle contrast	-0.80386

One can see almost high positive correlation between average speckle contrast and fluorescence emission (excited at 365 nm, as well as negative high correlation between average speckle contrast and fluorescence emission (excited at 450 nm) and between both of fluorescence intensities.

IV Conclusion

Obtained results prove the possibility of using multimodal approach to evaluate metabolic activity and microcirculation rate by both spectroscopic and imaging methods.

Combined measurements of fluorescence intensity and speckle contrast demonstrate close dependence of cell metabolism on oxygen supply. This result shows the necessity of further development of methodology and criteria for optical diagnostic for future prospects of clinical implementation. More research are necessary to reveal the correlation more clearly and find out the coefficients, which determines it.

The experimental results of this study will be used for adjusting the techniques of FS and LSCI for application in standard minimally invasive surgery tools. Especially it is planned to integrate this techniques in a laparoscope. The addition of more spectroscopy (diffuse reflectance spectroscopy) and imaging (fluorescence imaging, hyperspectral imaging) methods for further studies seems promising as well.

V Acknowledgements

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